

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(A) a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 14, wherein the polypeptide possesses a ceramidase activity;

(B) a nucleotide sequence comprising SEQ ID NO: 15, wherein said nucleotide sequence encodes a polypeptide possessing a ceramidase activity;

(C) a nucleotide sequence which hybridizes to the complement of the nucleotide sequence of SEQ ID NO: 15 ~~(A) or (B)~~ under stringent conditions, wherein said stringent conditions comprise 7% PEG 6000 containing 10% SDS solution at 60°C, and washing three times with 2xSSC containing 0.1% SDS for 15 minutes at 60°C ~~of 6 x SSC containing 0.5% SDS, 5 x Denhardt's, and 100 µg/ml salmon sperm DNA at 50°C~~, wherein said nucleotide sequence encodes a polypeptide having activity of hydrolyzing any one of substances selected from the group consisting of (i) N-Lauroylsphingosine, (ii) N-Palmitoylsphingosine, (iii) N-Stearoylsphingosine, (iv) N-Palmitoylsphinganine, (v) N-Stearoylsphinganine, and (vi) 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-

yl)amino)dodecanoyl)sphingosine ~~possessing a ceramidase~~
activity; and

(D) a nucleotide sequence different from the nucleotide sequence of ~~any one of the above (A) to (C)~~ due to via degeneracy of the genetic code, wherein said nucleotide sequence encodes a polypeptide having activity of hydrolyzing any one of substances selected from the group consisting of

(i) N-Lauroylsphingosine, (ii) N-Palmitoylsphingosine,
(iii) N-Stearoylsphingosine, (iv) N-Palmitoylsphinganine,
(v) N-Stearoylsphinganine, and (vi) 12-((N-(7-nitrobenz-2-oxa-
1,3-diazol-4-yl)amino)dodecanoyl)sphingosine ~~possessing a~~
~~ceramidase activity.~~

2. (Currently Amended) The nucleic acid according to claim 1, wherein the ceramidase activity of the polypeptide is detected by the following steps:

(a) incubating an expression product in a reaction mixture comprising 550 pmol of 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl)sphingosine and 1.0%(W/V) sodium cholate in 20 μ l of 25 mM Tris-hydrochloric acid buffer (pH 7.5) at 37°C for 30 minutes; and

(b) detecting the formation ~~generation~~ of a 12-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl acid in the reaction.

3. (Currently Amended) The nucleic acid according to claim 1 or 2, wherein the polypeptide exhibits at least the following characteristics:

(i) action of hydrolyzing ceramide to generate sphingoid and a fatty acid;

(ii) substrate specificity of hydrolyzing N-acylsphingosine, but not acting on galactosylceramide, sulfatide, Galb1-3GalNAcb1-4(NeuAca2-3)Galb1-4Glcbl-1'Cer (GM1a) GM1a, and sphingomyelin;

(iii) optimum pH of from 7.0 to 8.0; and

(iv) wherein incubation ~~no lowering of activity when treated~~ in 20 mM Tris-hydrochloric acid (pH 7.5) containing 0.1% polidocanol at 37°C for 24 hours does not decrease activity of said polypeptide, whereas incubation in 20 mM Tris-hydrochloric acid (pH 7.5) containing 0.1% polidocanol, ~~but lowering of activity to about 30% by a treatment~~ at 60°C for 1 hour decreases activity of said polypeptide to about 30%.

4. (Previously Presented) A recombinant DNA comprising the nucleic acid of claim 1.

5. (Previously Presented) An expression vector comprising the nucleic acid of claim 1 or the recombinant DNA of claim 4.

6. (Currently Amended) A transformed cell ~~transformant~~ comprising the expression vector of claim 5.

7. (Previously Presented) A method for producing a polypeptide possessing a ceramidase activity, comprising the steps of .

culturing the transformant of claim 6 under conditions appropriate for expression of the polypeptide, and

collecting a polypeptide possessing a ceramidase activity from the resulting culture.

8. (Previously Presented) An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 14, wherein said polypeptide possesses a ceramidase activity.

9. (Previously Presented) An isolated polypeptide possessing a ceramidase activity, wherein said polypeptide is encoded by the nucleic acid of claim 1.

10. (Currently Amended) The polypeptide according to claim 8 or 9, wherein the ceramidase activity is detected by the following steps:

(a) incubating an expression product in a reaction mixture comprising 550 pmol of 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl)sphingosine and 1.0%(W/V) sodium cholate in 20 μ l of 25 mM Tris-hydrochloric acid buffer (pH 7.5) at 37°C for 30 minutes; and

(b) detecting the formation ~~generation~~ of a 12-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl acid in the reaction.

11. (Currently Amended) An isolated ~~antisense~~ DNA which is complementary to the nucleic acid of claim 1.

12. (Currently Amended) An isolated ~~antisense~~ RNA which is complementary to the nucleic acid of claim 1.

13. (Currently Amended) An expression vector comprising the ~~antisense~~ DNA of claim 11.

14-19. (Cancelled).

20. (Currently Amended) A method of controlling an amount of ceramide in a cell ~~and/or in a tissue~~, comprising the step of introducing the nucleic acid of claim 1 or a complementary ~~an antisense~~ nucleic acid thereof into the cell ~~and/or in a tissue~~, thereby controlling the amount of ceramide in the cell ~~and/or in a tissue~~.

21. (Cancelled).